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DOI: <https://doi.org/10.1016/j.actatropica.2014.04.030>

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Journal Article

Accepted Version

Originally published at:

Sultanov, A; et al; Torgerson, P R (2014). Epidemiology of fishborne trematodiasis in Kazakhstan. *Acta Tropica*, 138:60-66.

DOI: <https://doi.org/10.1016/j.actatropica.2014.04.030>

Epidemiology of Fish Borne Trematodiasis in Kazakhstan.

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Keywords: Opithorchis, food born trematodes, epidemiology, Zoonosis, Kazakhstan

Abstract

Fish borne trematodes are an important cause of morbidity in Kazakhstan. The number of human cases of opisthorchiidosis (infection with parasites of the family opisthorchiidae) reached a peak of 2521 recorded cases (17 cases per 100,000) in 2002 with a gradual decline to 1225 cases (7.4 cases per 100,000) in 2011. Most human cases are found in the north and north east part of Kazakhstan in areas drained by the Irtysh River and its tributaries. A further focus is found in the north west in the Ural river basin in the European part of Kazakhstan. The most common occupations of patients with opisthorchiidosis included the manual labourers, those employed in the home or unemployed. Necropsy investigations of village dogs in an endemic region revealed 37 of 51 (72%) village dogs infected with either *Opisthorchis felineus* or *Methorchis bilis*. Likewise an investigation of 242 cats consisting of strays, village, suburban and city cats revealed 79 (33%) animals infected with *O. felineus*. Higher prevalences were seen in strays and village cats compared to suburban cats. No urban cats, which lived in apartments, were found to be infected. Other important zoonoses included *Echinococcus granulosus*, detected in 2 of the 51 necropsied village dogs and *E. multilocularis* was found in 2 out of 124 necropsied stray cats. Investigations of locally caught fish revealed 10 of 107 (9%) roach (*Rutilus rutilus*), 49 of 68 (72%) ide (*Leuciscus idus*) and 2 of 79 (2.5%) bream (*Abramis brama*) infested with trematode metacercariae. No metacercariae were found in 609 crucian carp (*Carassius carassius*), 35 tench (*Tinca tinca*), 79 carp (*Cyprinus carpio*), 46 perch (*Perca fluviatilis*) or 20 zander (*Sander lucioperca*).

Introduction

Food borne trematodes are a number of parasitic species transmitted by the consumption of undercooked, usually aquatic products. (Keiser and Utzinger, 2009) and infections with such trematodes cause a high global burden of disease. Over 56 million people are infected with food borne trematodes, 7.9 million with severe sequelae. Over 7000 deaths per year can be attributable to infection with foodborne trematodes (Fürst et al., 2012). The World Health Organization also recognises food borne trematode infections as a neglected tropical disease (World Health Organization, 2010)

Kazakhstan is the largest landlocked country in the world with the western most district being geographically part of Europe and eastern districts bordering China. The country has an area of 2.7 million square kilometers and a population of approximately 17 million, of which 46% is rural, and population density of 5.9 per km². Agriculture represents 5% of economic output. The climate is continental, with warm summers and colder winters. Precipitation varies between arid and semi-arid conditions.

Opisthorchis felinus is known to be endemic in the new independent states of the former Soviet Union (Ilyinskikh , 2002; Kochetkov et al., 2010; Syskova et al., 2001:). The first intermediate hosts of *O. felinus* are species of freshwater snails from the genus *Bithynia* (Brusentsov et al., 2013; Mordvinov et al., 2012). Fish of the Cyprinidae family serve as the second intermediate hosts (Pozio et al., 2013). Metacercariae, the encysted and resting stage of a fluke larva, accumulate in muscles of susceptible fish. When final hosts ingest raw fish, the young trematode escapes from the metacercarial cyst and migrates through the common bile duct into the bile duct and gall bladder, where it develops into an adult. Mature flukes live in the liver of definitive hosts (fish-eating mammals, including humans). Human infections occur by consuming raw or undercooked fish infested with metacercariae.

O. felinus affects 1.6 million people (Yossepowitch et al., 2004). In humans, acute *O*

felineus infection may result in hepatitis-like symptoms including fever, abdominal pain, nausea and emesis. In chronic infections, inflammation, fibrosis and obstruction of the biliary tract, liver abscesses, pancreatitis and suppurative cholangitis may occur (Keiser and Utzinger, 2009). The carcinogenicity of chronic *O. felineus* infections is yet to be proven. However, available epidemiological and clinical data in humans and animals suggest that *O. felineus* can be the cause of neoplasia. There is a clear increase in the incidence of cholangiocarcinoma in endemic areas of Siberia. In Russia, the highest incidence of bile duct cancer in humans was documented in the same area (i.e., Tyumen Region) with the highest incidence of *O. felineus* infection in humans (Mordvinov et al., 2012) and previous estimates of the health impact of this parasite have assumed that infection carries the same relative risk of carcinogenesis as *O. viverrini* infection (Parkin, 2006).

The parasite has been reported to be endemic in Aktobe, Karadanda, Pavlodar, Akmola and Kostanay districts (World Health Organization, 1995). Wild caught fish are commonly consumed raw or slightly salted. Consequently, there have previously been a number of reports in the Russian literature on opisthorchosis in humans, dogs and cats. Pantiukhov and Vinogradova (1963) described up to 100% of cats and 70% of dogs infected in the Pavlodar and Semipalatinsk regions. In addition there were a large number of human cases of infection diagnosed in Pavlodar which were associated with the habit of eating salted dried fish. In some villages along the Irtysh river human prevalences of 42% have been recorded in the past (Terentyeva 1985) and Zhumabekova and Kamyeshyeva (2008) reported that the annual incidence of human opisthorchosis had increased from under 10 per 100,000 to over 140 per 100,000 between 1995 and 2006 in the Kashir district (population 21,000). Studies of fish from the Irgiz and Turgay river basins have previously found the prevalence of infection in fish of up to 50% (Khokhalkova 1957). Older data has indicated foci of opisthorchosis in the Karaganda and Tamirtau regions (Kreptogorskoy 1932). More recently there is a report of 4.5% of Prussian carp (*Carassius gibelio*) recovered from the Baikon lakes, Kashir district in the Pavlodar Oblast in north Eastern Kazakhstan were infected with metacercariae of *Opisthorchis felineus*.

(Zhumabekova and Kamyeshyeva 2008).

The objective of this study was to report the incidence of infection in humans in Kazakhstan. This study reports the numbers of officially reported cases of human opisthorchiidosis in Kazakhstan between 1997 and 2011 and analyses the trends in the disease. We also give details of the geographical origin and occupations of cases since 2007. Therefore a second objective to this study was to investigate the prevalence of zoonotic trematodes in potential definitive and intermediate hosts in one of these endemic districts, namely Akmola district. Consequently, we report surveillance results from domestic definitive and intermediate hosts of *O. felineus* from the northern part of Kazakhstan.

Materials and Methods

Human data

Human faecal samples are supplied by patients who are suspected clinical cases of opisthorchiidosis (or indeed possibly other diseases) and the samples submitted to regional diagnostic laboratories by the patients' physician between 1997 and 2011. The Kato-Katz technique and/or the ether sedimentation technique (as described by WHO 1991) was used for routine diagnosis of human helminthoses. All data is sent to the Scientific Practical Center of Sanitary and Epidemiological Expertise and Monitoring, Almaty. From these records data human opisthorchiidosis are reported together with details of the origin of the case and occupation.

Dogs

A total of 51 dogs from villages were necropsied from rural areas of Akmola district. These dogs were from a village environment and euthanised at the request of their owners and hence were a convenience sample. All helminth parasites were removed from the gastrointestinal tract and liver

and identified morphologically, with the use of microscopy where necessary. The bile ducts were incised specifically to find any infection with hepatic trematodes. Total parasite counts of each species identified were recorded.

Cats

We undertook necropsy of 242 cats from rural areas of Akmola district and from Astana. Astana, the capital of Kazakhstan is completely surrounded by Akmola district. These cats were urban cats living in apartments (n=56), suburban cats (n=85), village cats (n=128) or stray cats (n=72). All cats were humanely euthanised by the request of their owner, and were also inevitably a convenience sample. All helminth parasites were recovered from the gastrointestinal tract and liver and identified.

Fish

In total we investigated 1043 specimens of freshwater fish representing 8 species. Fish were purchased from various markets and shops in Astana or Akmola district, or caught directly from the Korgaldjin lakes (also in the Akmola district). The species of freshwater fish examined were carp (*Cyprinus carpio*), crucian carp (*Carassius carassius*), roach (*Rutilus rutilus*), tench (*Tinca tinca*), ide (*Leuciscus idus*), rudd (*Scardinius erythrophthalmus*), bream (*Abramis brama*), perch (*Perca fluviatilis*) and zander (*Sander lucioperca*). Visual inspection and microscopy was performed to the internal organs of the body cavity, the eye and the edible parts of the fish's body (muscles). The skin was separated from underlying subcutaneous tissue. Thin layers of muscle of no more than 2-3mm thickness were scraped off with a sharp scalpel or cut and squeezed between glass slides and examined microscopically. To identify the larval stages of the parasites in the muscles of fish examined by the compressor. The parasites in fish were identified microscopically according to

Bayer and Shulman (1984).

The studies on dogs, cats and fish were undertaken between 2008 and 2010.

Statistical Analysis

Trends in the numbers of human cases were examined by χ^2 for trend. Abundances and prevalences and their confidence intervals were examined by generalized linear models using R (R Core Team, 2013) .

Ethical statement

All euthanised animals were at the specific request of their owners with a further request to dispose of the animal carcass with permission given for collection of any relevant samples for surveillance purposes. All human data was from routine collected surveillance data and anonymised so that no individual patient details can be identified.

Results

Human cases

The numbers of cases of human opisthorchiidosis (ie the of patients from which opisthorchiid type eggs were isolated) ranged from a maximum of 2521 (annual incidence of 17 cases per 100,000) in 2002 to a minimum of 1225 (7.4 cases per 100,000) in 2011. There is a clear trend after 2002 with a declining number of cases (χ^2 for trend, $p < 0.001$) (figure 1). Nearly all cases are in the north of the country with a particularly high incidence seen in Pavlodar District in the north east of the country (figure 2). There was also a focus in the European part of Kazakhstan in the west Kazakhstan district. Patients had a variety of occupations, but with many cases being in people who were not

employed or worked in the home or as manual labourers (figure 3). For the years 2006-2011 there was also data available for the numbers of cases in children 14 years or under. This varied from 69 of 1252 cases (5.5%) in 2010 to 124 of 1525 cases in 2008. Although there was some variation in the proportion of cases that were children, there was no significant trend over this 6 year period (χ^2 for trend, $p=0.15$).

Dogs

Table 1 summarizes the parasites recovered from the necropsy of 51 village dogs. *O. felinus* was recovered from 35 dogs with a mean abundance of 30 parasites per dog (Table 1). Two dogs were infected with single specimens of *Methorchis bilis*. Of further note is that 2 dogs were also infected with *Echinococcus granulosus*. All parasites in dogs had an overdispersed distribution regardless of diagnostic method, except *Taenia* spp, which appeared to be randomly (Poisson) distributed.

Cats

O. felinus was found in village, suburban and stray cats, but not in cats confined to apartments (Table 2). Village cats had the highest prevalence with 44 of 65 cats infected (68%). This was significantly higher than either stray cats or suburban cats (Fisher test $p < 0.001$), with no difference between the latter two groups having 24% and 20% prevalences respectively. Two stray cats were found to be infected with *E. multilocularis*.

Fish

Three species of freshwater fish were found to be infected with Opisthorchiidae spp (Table 3). A total of 49 Ide (*Leuciscus idus*) out of 68 examined (72%) were infected. In contrast just 10 of 107 roach (*Rutilus rutilus*) and 2 of 79 bream (*Abramis brama*) were infected.

Discussion

This study indicates that human opisthorchiidosis is an important public health issue in the north of Kazakhstan, although the data indicates a slowly declining trend in the numbers of reported cases. Most human cases were seen in the northern and northeastern regions which are drained by the River Irtysh and its tributaries the Ishim and Tobol Rivers. A further focus was seen in the northwest of the country which is drained by the Ural River, considered to be the Europe-Asia boundary. This report gives further important information on the epidemiology of fish borne trematodes in Kazakhstan in addition to that previously reported in the Russian literature. Information regarding the detailed clinical symptomatology, such as duration or presenting signs, of the patients from which samples were taken is unavailable. It is possible that some of the cases were incidental findings when the referring physician suspected other disease. However, the referring physician is likely to have instigated appropriate treatment for trematodiasis on receipt of the laboratory report. Nevertheless, regardless of the reason the referring physicians submitted the faecal samples, the data gives the annual incidence of laboratory confirmed cases of opisthorchiidosis in Kazakhstan.

The study in fish indicates infection of locally caught freshwater fish is common with the ide in particular having a high prevalence, with roach and bream also being infected. In Siberia, ide, roach, beluga (*Leucaspis delineatus*), bream, dace and minnow (subfamily Leuciscinae) are known to be suitable intermediate hosts of Opisthorchiidae (Karpenko et al., 2008; Mordvinov et al., 2012), with ide and beluga predominating with prevalences of over 40% in some localities. In contrast in a recent study in Italy, the only species of fish found to be infected was the tench (De Liberato et al., 2011) which was not found to be infected in the present study.

Village dogs have a high prevalence of infection with *O. felinus* and could be infected from

the same fish that are infecting humans. This is also true of rural cats. Suburban, village and stray cats were all infected with *O. felineus*. Village cats had the highest prevalence – approaching 68% whilst village dogs had a prevalence of 69%. Urban cats in contrast had no infection and thus may have less access to raw fish. The contribution of domestic carnivores in maintaining the cycle is unknown. It is possible that faeces from infected cats and dogs contaminate water courses allowing infection of the first intermediate host. This would then allow onward transmission to fish and hence to humans. Previous reports have indicated that cats and dogs are frequent definitive hosts in Siberia (Mordvinov et al., 2012) which is consistent with our findings. In particular prevalences of 61.5% have been reported in cats and 11.6% of dogs from settlements along the Irtysh River in south west Siberia (Karpenkon et al., 2008). However a number of wild carnivores such as foxes (Schuster et al., 1999) mink (Shimalov and Shimalov, 2001) otter (Shimalov et al., 2000) pig and other mammals (Mordvinov et al., 2012) are also suitable definitive hosts. Transmission of fish borne trematodes appears to be density independent (Boerlage et al., 2014) and thus it is difficult to come to conclusions about the relative contributions of different hosts species for transmission. In Italy the only definitive host found to be infected were domestic cats during an investigation following an outbreak of human opisthorchosis in central Italy (De Liberato et al., 2011)

The necropsy studies confirmed that the main trematode infecting dogs and cats in this regions was *O. felineus*, with two specimens of *Methorchis bilis* recovered from dogs. Coprological studies based on egg identification only are not able to distinguish between these two species and it is known that both species are endemic across much of this region (Mordvinov et al., 2012). As the infections in humans were made by morphology of eggs in the faecal samples, it is not possible to confirm whether the human Opisthorchiidosis were due to *O. felineus* or *M. bilis*. Furthermore, it is not possible to rule out the presence of intestinal flukes as they are frequently misdiagnosed as *Opisthorchis* spp due to similarities in egg morphology (see for example Chai et al, 2013). However, small intestinal flukes were not observed in the necropsy studies of dogs and cats despite the careful

examination of the intestines necessary to demonstrate the presence of the small *Echinococcus* spp. In neighbouring Siberia it is believed that many cases of human cases of Opisthorchiidosis may be caused by *M. bilis* and possibly other trematodes in addition to *O. felineus* (Karpenkon et al., 2008). Likewise the metacercariae of the two species are morphologically indistinguishable (Mordvinov et al., 2012) and thus the fish examined could have been infected by either parasite. Nevertheless, the public health data and the study on domestic definitive hosts and freshwater fish used for human consumption indicate that there is major transmission of fish borne trematodes in this region of Kazakhstan which has an impact on human health burden.

There appears to be a trends in the decrease in the numbers of reported human cases. With the available data it is only possible to speculate as to the reasons for this. Kazakhstan has one of the fastest growing economies in the world so a rapid improvement in living standards and improvements in government public health services are likely to decrease the incidence of any transmissible disease.

In neighboring regions of Russia human infection has been described in several studies. In the Tomsk region annual incidence of disease increased from 495 per 100,000 to 649 per 100,000 between 1997 and 2006 (Mordvinov et al., 2012). Surveillance data in Tomsk Oblast has also indicated a high prevalence of infection with over 11% of urban inhabitant and 32% of rural inhabitants infected (Orgorodova et al. 2007). In other areas of Siberia such as Irkutsk region the incidence appears to be somewhat lower and stable at about 3.4 cases per 100,000 per year between 2001 and 2011 (Kupriyanova and Timoshenko, 2012)

This study also illustrates that dogs from this region have a low prevalence of *E. granulosus* and is consistent with fewer human cases of CE are seen in northern Kazakhstan compared to southern or western districts. (Torgerson et al., 2002). *E. multilocularis* was also isolated from two cats. Although cats have been reported previously to be infected with this parasite (for example Petavy et al., 2000), experimentally they appear to be poor hosts and hence are unlikely to make any

significant contribution to transmission (Kapel et al., 2006).

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Legend for Figures

Figure 1. Numbers of laboratory diagnosed cases of human opithorchiidosis reported in Kazakhstan between 1997 and 2011.

Figure 2. Geographical distribution of human opithorchiidosis in Kazakhstan. Annual incidence of laboratory diagnosed cases per 100,000 between 2010-2011.

Figure 3. Numbers of human cases of opithorchiidosis in Kazakhstan by occupation in 2011
Occupation that could not be specified is given as “other”.

Figure 1

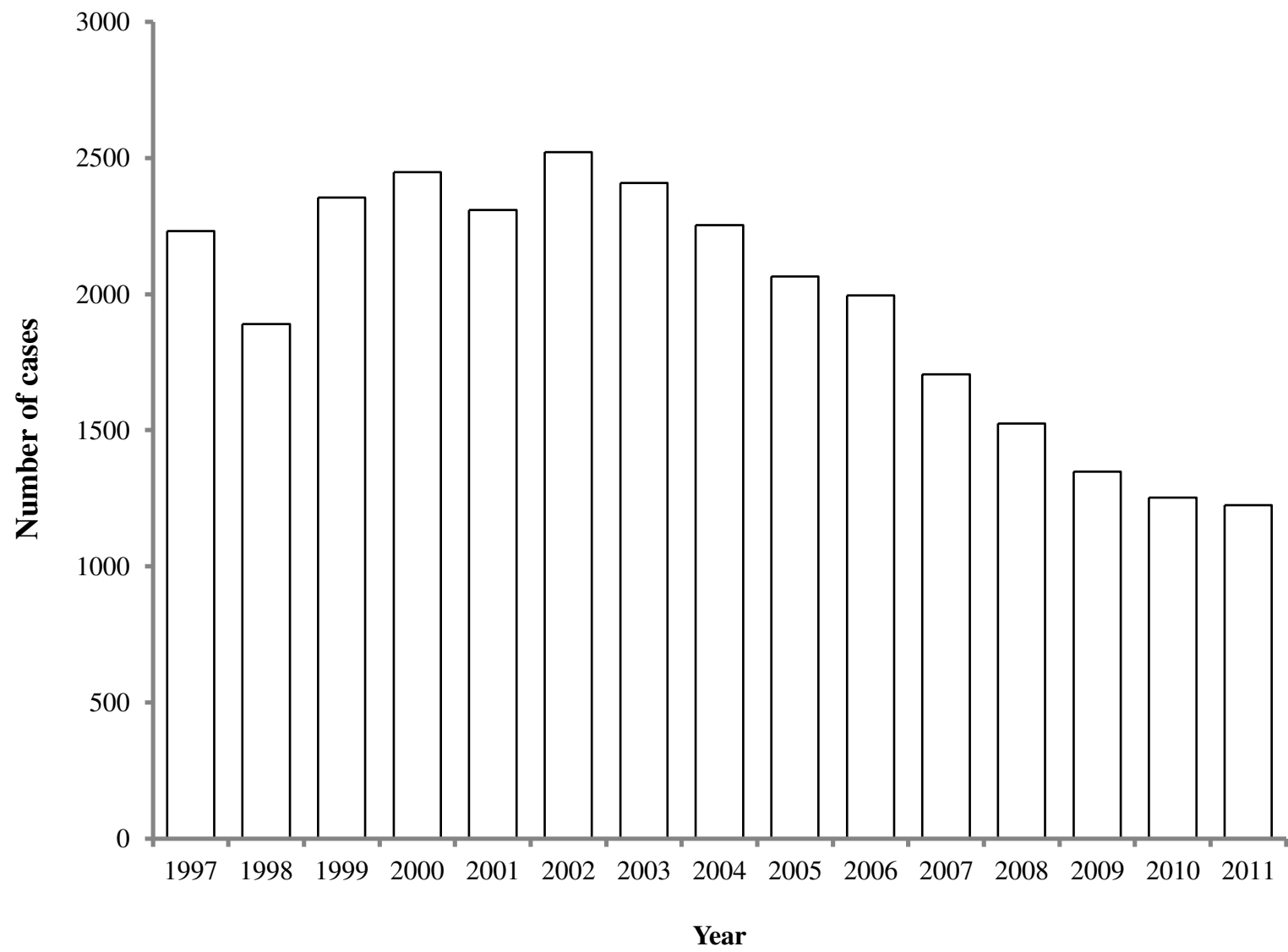


Figure 2 revised

Area for dog, cat and fish studies

Pavlodar region

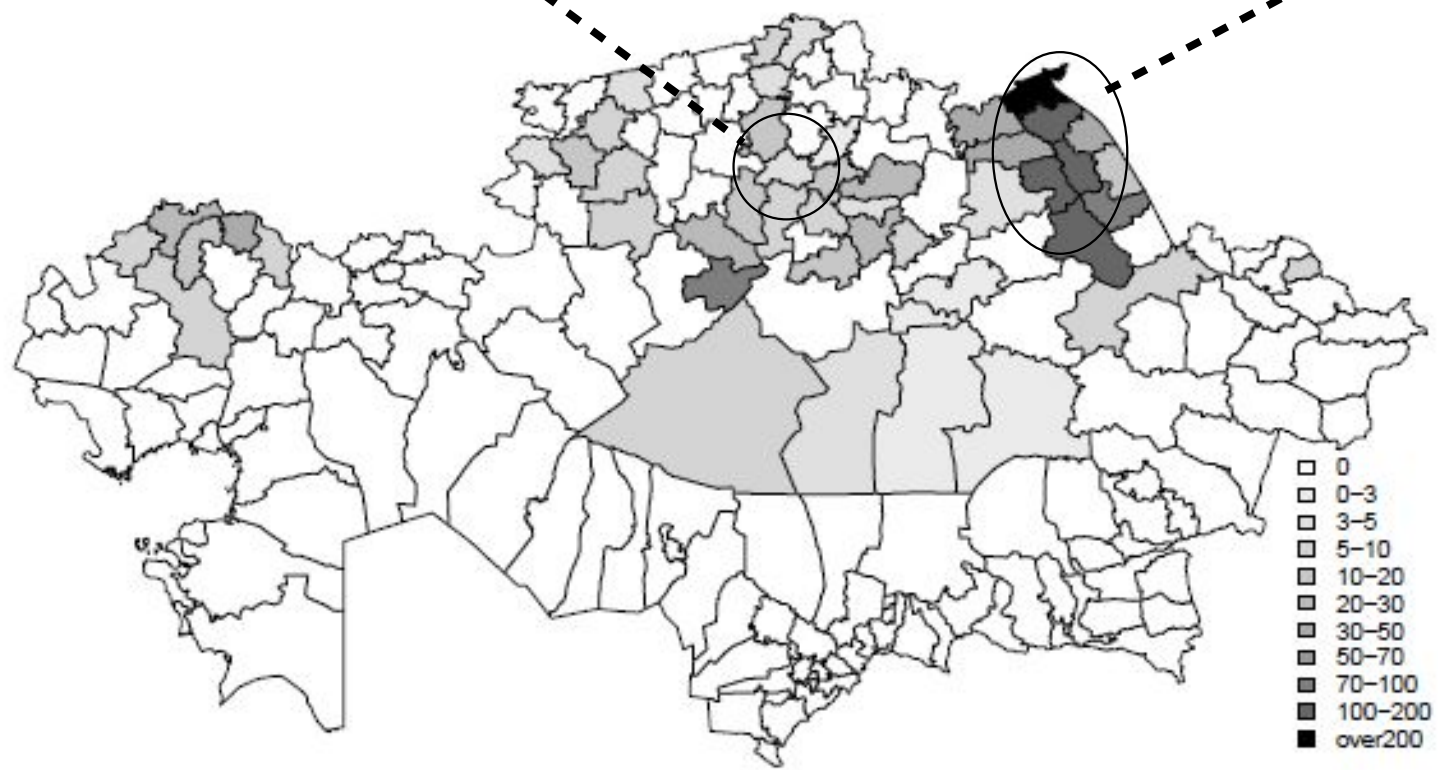


Figure 3

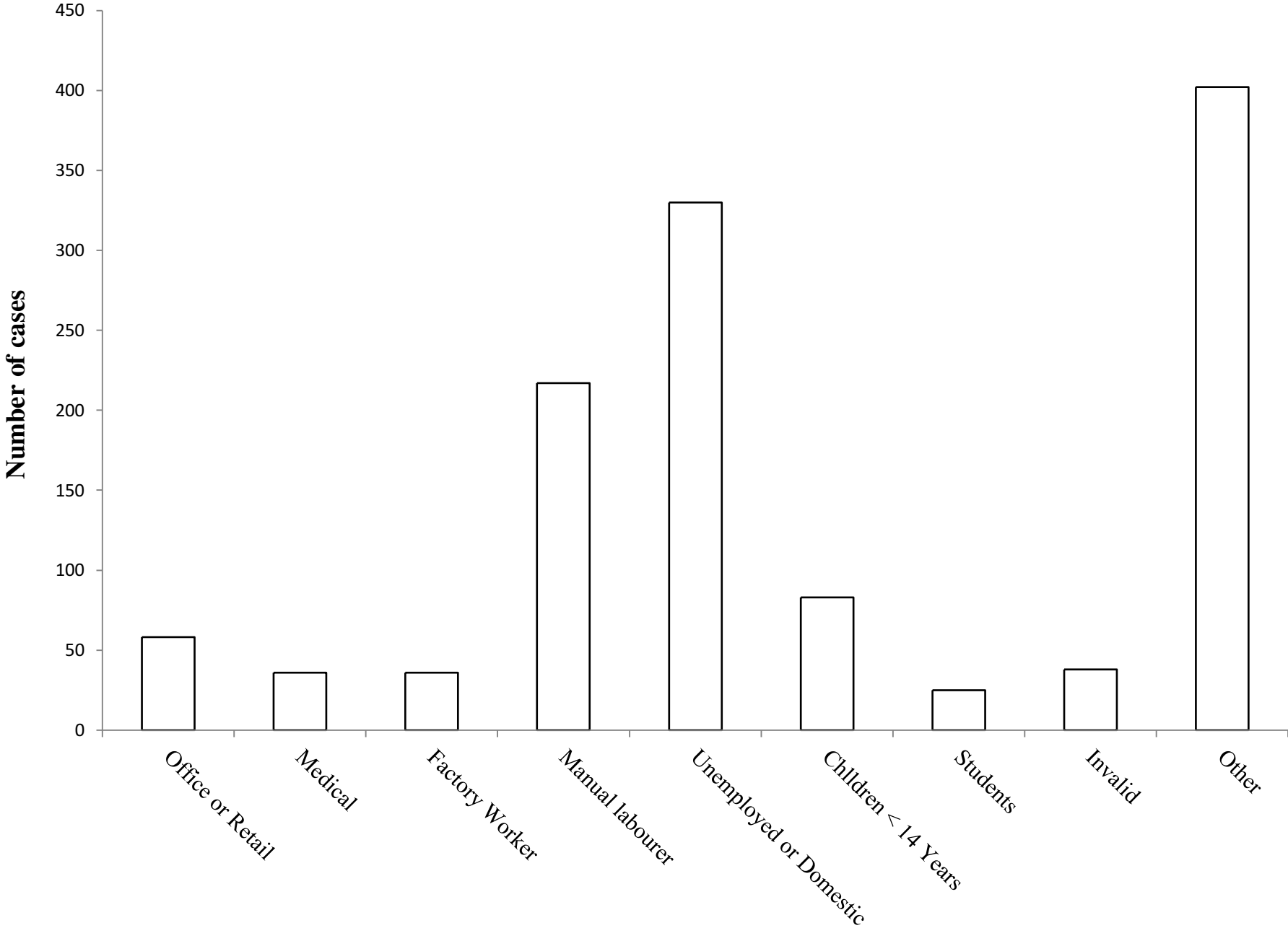


Table 1

Table 1. Numbers of dogs infected from a sample of 51 village dogs and the abundance of each parasite species found.

Parasite Recovered	No. infected	Mean Abundance (confidence intervals)
<i>Dipylidium caninum</i>	17	1.82 (0.991-3.81)
<i>Echinococcus granulosus</i>	2	3.45(0.496-11.34)
<i>Taenia</i> spp	3	0.078 (0.020-0.267)
<i>Toxascaris leonina</i>	15	2.00 (1.14-3.22)
<i>Toxocara caninum</i>	39	5.26 (3.74-7.63)
<i>Uncinaria</i> spp	1	0.020 (0.001-0.086)
<i>Methorchis bilis</i>	2	0.040 (0.007-0.120)
<i>Opisthorchis fileneus</i>	35	30.1 (19.0-51.5)

Table 2. Numbers of cats infected and the abundance of each parasite species found

		Type of cat and number of cats examined			
		Cats in city apartments (n=25)	Cats from the suburbs (n=28)	Village Cats (n=65)	Stray Cats (n=124)
<i>Dipylidium caninum</i>	Abundance	0.165 (0.061-0.437)	4.75(3.36-6.87)	4.31 (3.43-5.41)	2.13(1.83-2.47)
	No Infected	1	22	51	103
<i>Joyeuxiella rossicum</i>	Abundance	0	0.616(0.035-2.67)	0	0.12(0.037-0.28)
	No Infected	0	1	0	4
<i>Echinococcus multilocularis</i>	Abundance	0	0	0	2.92(0.440-9.32)
	No Infected	0	0	0	2
<i>Taenia taeniaformis</i>	Abundance	0	4.32(2.97-6.47)	2.17 (1.83-2.54)	2.97(2.62-3.40)
	No Infected	0	21	54	120
<i>Mesocestoides lineatus</i>	Abundance	0	0	0.231 (0.032-0.783)	0.13(0.081-0.212)
	No Infected	0	0	2	4
<i>Toxascaris leonina</i>	Abundance	1.16 (0.81-1.67)	0	0.492 (0.138-1.21)	0.223(0.083-0.450)
	No Infected	5	0	4	6
<i>Toxocara cati</i>	Abundance	2.84 (1.50-5.34)	6.54(4.61-9.55)	3.49 (2.87-4.24)	1.66(1.41-1.95)
	No Infected	9	24	52	93
<i>Opithorchis felineus</i>	Abundance	0	38.2 (10.8-92.8)	81.6 (50.0-146)	18.2(8.96-46.1)
	No Infected	0	5	44	30

Table 3

Table 4. Parasitic infection of various freshwater fish species

Species	Number Examined	Number infected								
		Myxosporea spp.	Microsporidi a spp.	<i>Diplostomum</i> sp., larvae	Dilepididae spp	<i>Ligula</i> spp	Opisthorchiidae spp	<i>Khawia</i> <i>sinensis</i>	<i>Philometroides</i> <i>cyprini</i>	<i>Tetracotyle</i> <i>spp</i>
Crucian carp <i>Carassius carassius</i>	609	34	10	75	11	0	0	0	0	0
Roach <i>Rutilus rutilus</i>	107	0	1	4	0	2	10	0	0	0
Tench <i>Tinca tinca</i>	35	0	2	0	1	0	0	0	0	0
Ide <i>Leuciscus idus</i>	68	0	0	29	0	0	49	0	0	0
Carp <i>Cyprinus carpio</i>	79	0	0	11	0	0	0	1	1	0
Bream <i>Abramis brama</i>	79	0	0	15	0	0	2	0	0	1
Perch <i>Perca. fluviatilis</i>	46	0	0	3	0	0	0	0	0	0
Zander <i>Sander lucioperca</i>	20	0	0	1	0	0	0	0	0	0

Epidemiology of Fish Borne Trematodiasis in Kazakhstan.

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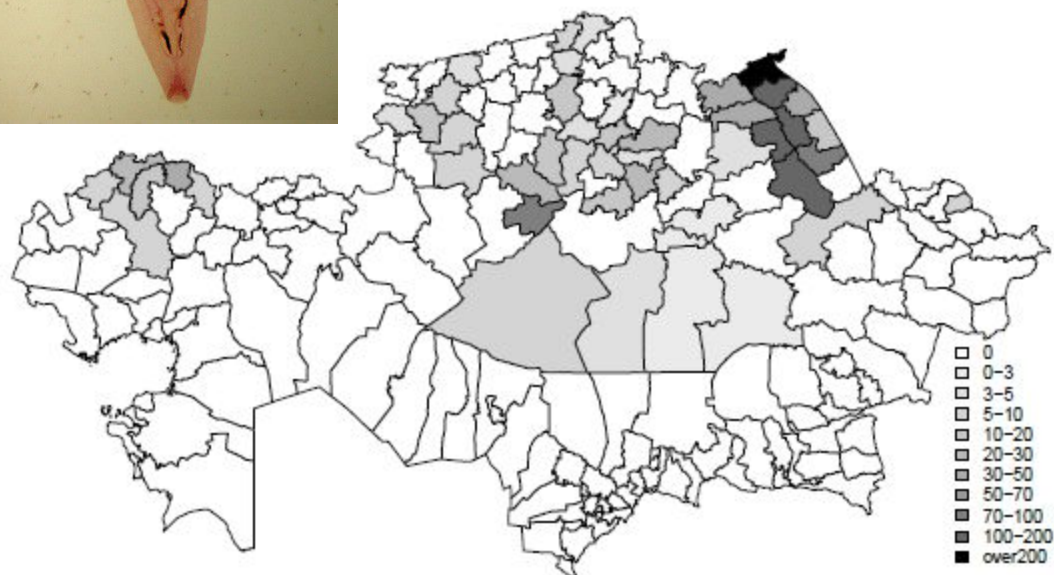
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Graphical abstract

Fish borne trematodiasis is an important public health problem in Kazakhstan, particularly in the north of the country.



Highlights

- Fish borne trematodiasis is an important public health problem in Kazakhstan
- Approximately 1200 human cases are recorded each year
- Annual incidences can be as high as 200 per 100,000 in some localities
- Cats and dogs are commonly infected definitive hosts in north Kazakhstan
- Freshwater fish for human consumption are frequently infected with metacercariae